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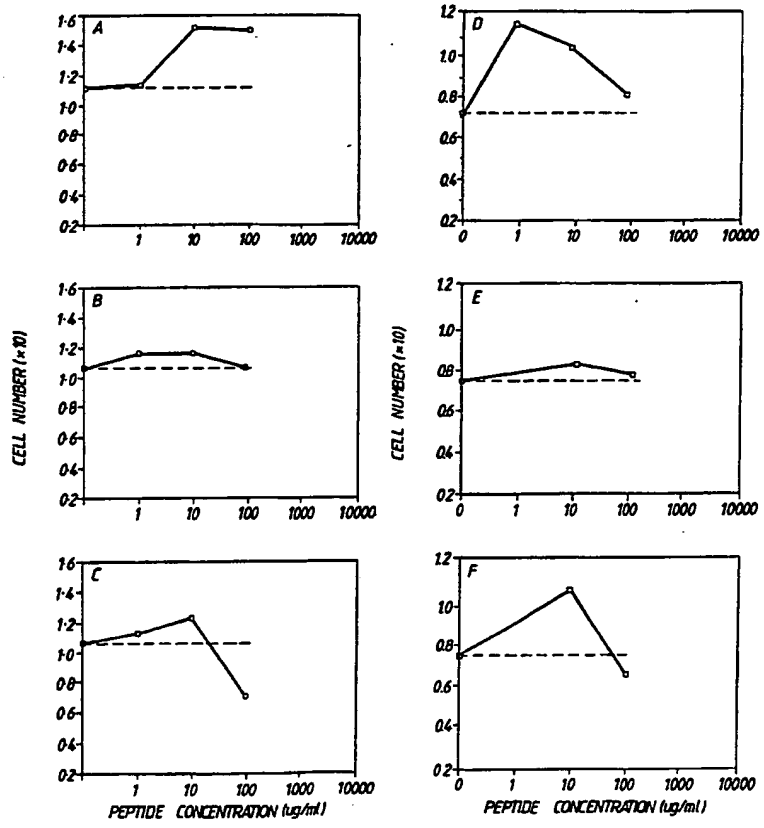
(57) Abstract

The present invention relates to Intercellular Adhesion Molecule-1-like peptides, analogues thereof and antibodies thereto, to pharmaceutical compositions comprising same and the use thereof to inhibit or reduce infection of mammalian cells by retroviruses, and in particular to inhibit or reduce infection of human cells by HIV or its variants.



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INHIBITION OF VIRAL INFECTION USING INTERCELLULAR ADHESION MOLECULE-1-LIKE PEPTIDES AND/OR ANALOGUES THEREOF.

The present invention relates to Intercellular Adhesion Molecule-1-like peptides, analogues thereof and antibodies thereto, to pharmaceutical compositions comprising same and the use thereof to inhibit or reduce infection of mammalian cells by retroviruses, and in particular to inhibit or reduce infection of human cells by HIV or its variants.

The specific interaction between the two cell surface glycoproteins, Intercellular Adhesion Molecule-1 (hereinafter referred to as "ICAM-1") and Leukocyte Function Associated antigen-1 (hereinafter referred to as "LFA-1") has been shown to be a major adhesive mechanism for haemopoietic and lymphoid cells (Wawryk et al., 1989). Studies using both ICAM-1 and LFA-1 antibodies have shown that the LFA-1/ICAM-1 interaction is critically involved in a wide variety of adhesion-dependent leukocyte functions (Wawryk et al., 1989). Cytokines involved in the "inflammatory" response, IFN-γ, TNFα and IL-1, induce expression of ICAM-1 (Pober et al., 1987: Dustin et al., 1988; Boyd et al., 1989a,b; Campbell et al., 1989). These critical roles of ICAM-1 in leukocyte migration at sites of inflammation, T-cell activation and cell-cell interactions involved in both regulatory and effector aspects of the inflammatory response, place this receptor amidst the same cells, activated T-cells and monocytes/macrophages, which are susceptible to human immunodeficiency virus (hereinafter referred to as HIV) infection.

HIV has a selective tropism for cells expressing CD4 (Dalgleish et al., 1984) present on T-lymphocytes and monocyte/macrophages and requires these cell populations to be activated for virus replication (McDougal et al., 1985). The infection process involves binding of infectious virus or virus-infected cells, through the surface glycoprotein involved in binding, to the CD4 receptor on susceptible cells. After binding, entry involves fusion of the virus or virus-infected cell to the susceptible cell at least via the HIV transmembrane glycoprotein (Kowalski et al., 1987), possibly with other molecules being involved.

After infection "in vitro", two types of HIV-induced cytopathology are observed. The first involves fusion of the plasma membrane of an infected cell with the plasma membranes of other CD4+ cells. Multiple rounds of cell-cell fusion result in the formation of giant, multinucleated cells which are generally observed to be in some stage of "balloon" degeneration (Lifson et al., 1986). This effect is called syncytium formation. The second type of HIV-induced cytopathology involves lysis of individual cells (Somasundaran and Robinson, 1988). Both of these mechanisms have been postulated to account for the loss of CD4+ cells "in vivo".

Recently, three papers described a possible role for LFA-1 and/or ICAM-1 in the HIV infection process (Hildreth and Orentas, 1989; Valentin et al., 1990, Gruber et al., 1991) following experiments using monoclonal antibodies directed against LFA-1 and/or ICAM-1.

In work leading up to the present invention, the possibility that ICAM-1 may be involved in HIV infection was investigated. In accordance with the present invention it was discovered that ICAM-1-like peptides are capable of inhibiting HIV-induced syncytium formation and virus production.

Accordingly, one aspect of the present invention is directed to ICAM-1-like peptides and to analogues thereof. By "ICAM-like peptides" as used in the specification and claims is meant a peptide, or a polypeptide, having an amino acid sequence substantially similar to, or identical with, a region of ICAM-1 and capable of reducing, inhibiting and/or interfering with retroviral replication in mammalian cells and in particular replication of HIV or its variants in human cells. An amino acid sequence substantially similar to a region of ICAM-1 includes sequences having greater than 70% homology and preferably greater than 80% homology with the selected ICAM-1 region.

The ICAM-1-like peptides may have an amino acid sequence identical with the corresponding sequence in ICAM-1 or may contain single or multiple amino acid additions, deletions and/or substitutions compared to the amino acid sequence of the particular region of ICAM-1. The peptides contemplated herein may be chemically synthesized such as by solid phase peptide synthesis or may be prepared by subjecting the ICAM-1 polypeptide to hydrolysis or other chemically disruptive processes whereby fragments of the molecule are produced. Alternatively, the peptides could be made <u>in vitro</u> or <u>in vivo</u> using recombinant DNA technology. In this case, the peptides may need to be synthesized in combination with other proteins and then subsequently isolated by chemical cleavage or the peptides may be synthesized in multiple

repeat units. Furthermore, multiple antigen peptides could also be prepared according to Tam (1988). The selection of a method of producing the subject peptides will depend on factors such as the required type, quantity and purity of the peptides as well as ease of production and convenience.

The use of these peptides in vivo may first require their chemical modification since the peptides themselves may not have a sufficiently long serum and/or tissue half-life. Such chemically modified ICAM-1-like peptides are referred to herein as "analogues". The term "analogues" extends to any functional chemical equivalent of an ICAM-1-like peptide characterized by its increased stability and/or efficacy in vivo or in vitro in respect of the ability to reduce, inhibit and/or interfere with HIV replication.

Analogues of ICAM-1-like peptides contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction

with $NaBH_4$.

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2, 3 butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivatisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphide with other thiol compounds; reaction with maleimide, malaic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuric-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides could be conformationally constrained by, for example, incorporation of C_α- and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The present invention, therefore, extends to peptides and/or polypeptides and functional and/or chemical analogues corresponding to regions of ICAM-1 and which are capable of reducing, inhibiting and/or interfering with retroviral (e.g. HIV) replication. Use of the term "ICAM-1-like peptide" in the specification and claims herein is intended to include all such amino acid and chemical analogues of ICAM-1-like peptides broadly described above. Furthermore, such analogues include ICAM-1-like peptides in tandem or multiple repeats wherein each repeat is the same peptide or a different

ICAM-1-like peptide. Such tandem or multiple repeat molecules may first need to be cleaved before becoming active. Additionally, the ICAM-1-like peptides may be a part of a larger molecule wherein the larger molecule may or may not be related to the native ICAM-1 polypeptide. Conveniently, however, larger fragments of ICAM-1 which include the preferred amino acid sequences are used.

In one preferred embodiment, the ICAM-like peptides correspond to amino acids in one or more of the amino acid regions 1 to 43, 460 to 507, 340 to 420 and/or 101 to 150 of ICAM-1. More preferably, the ICAM-like peptides correspond to amino acids in one or more of the amino acid regions 1 to 23, 367 to 394, 479 to 507 and/or 114 to 141 of ICAM-1. The foregoing amino acid positions are according to Simmons et al., (1988).

Even more preferably, the present invention provides inter alia the following ICAM-1-like peptides:

JF7B, having the amino acid sequence:
NAQTSVSPSKVILPRGGSVLVTC and its amino acid and chemical analogues;

JF9, having the amino acid sequence:
VLYGPRLDERDAPGNWTWPENSQQTPMC and its amino acid and chemical analogues;

JF10, having the amino acid sequence:
GGAPRANLTVVLLRGEKELKREPAVGEP and its amino acid and chemical analogues; and

JFI3A, having the amino acid sequence:
NRQRKIKKYRLQQAQKGTPMKPNTQATPP and its amino acid and chemical analogues.

The aforementioned ICAM-1-like peptides inhibit HIV infection. Although not intending to limit the present invention to any particular mode of action of these peptides, the subject peptides may act in one or more of the following ways:

(i) binding of the peptides to the receptor LFA-1 and thus preventing HIV binding to LFA-1;

(ii) binding of the peptides to HIV and thus preventing binding of HIV to ICAM-1; and/or

(iii) inhibition of HIV infection via to some other receptor due to blocking of the ICAM-1/LFA-1 interaction.

The mode of action may involve other mechanisms in addition or in place of those listed above but this in no way limits the scope of the present invention.

ICAM-1-like peptide JF7B displayed the most marked effect on HIV infection and syncytia formation and, hence, indicates the importance of the amino terminus of the ICAM-1 molecule for HIV replication. The amino terminus of ICAM-1 may, for example, interfere with fusion preventing LFA-1/HIV transmembrane glycoprotein interactions.

Accordingly, the present invention is also directed to ICAM-1-like peptides and/or their chemical analogues capable of interfering with HIV replication. In this regard, the subject ICAM-like peptides and/or their analogues may be useful in inhibiting, reducing and/or interfering with infection by pathogens via the entry process. One such example is the malaria parasite Plasmodium falciparum. The present invention, therefore, extends to the use of ICAM-1-like peptides or their

chemical analogues to inhibit or reduce infection by pathogens.

Amino acid analogues of JF7B contemplated herein include, but are not necessarily limited to, the following shown in Table 1:

Table 1

DERIVATIVES OF JF7B

AMINO ACID SEQUENCE	ICAM-1 SEQUENCE POSITION ACCORDING TO SIMMONS *et al.* (1988)
AQTSVSPSKVILPRGGSVLVTC	[2-23]
NAQTSVSPSKVILPRGGSV	[1-19]
AQTSVSPSKVILPRGGSV	[2-19]
NAQTSVSPSKVILPR	[1-15]
AQTSVSPSKVILPR	[2-15]
NAQTSVSPSKV	[1-11]
AQTSVSPSKV	[2-11]
NAQTSVS	[1-7]
AQTSVS	[2-7]
NAQTSV	[1-6]
AQTSV	[2-6]
NAQTS	[1-5]
AQTS	[2-5]
NAQT	[1-4]
AQT	[2-4]
NAQ	[1-3]
AQ	[2-3]

Amino acid analogues of JF9 contemplated herein include, but are not necessarily limited to, the following shown in Table 2:

Table 2

DERIVATIVES OF JF9	
AMINO ACID SEQUENCE	**ICAM-1 SEQUENCE POSITION ACCORDING TO SIMMONS et al. (1988)**
VLYGPRLDERD[X]PGNWTWPENSQQTPMC	[367-394]
VLYGPRLDERD[X]	[367-378]
PRLDERD[X]	[371-378]
PGNWT	[379-383]
ERD[X]PGNWT	[375-383]
PRLDERD[X]PGNWT	[371-383]
VLYGPRLDERD[X]PGNWT	[367-383]
GNWTWPENSQ	[380-389]
RD[X]PGNWTWPENSQ	[376-389]
PRLDERD[X]PGNWTWPENSQ	[371-389]
VLYGPRLDERD[X]PGNWTWPENSQ	[367-389]
DERD[X]PG	[374-380]
WPENSQQ	[384-390]
PENSQQTPMC	[385-394]

[X] is A or C

Amino acid analogues of JF10 contemplated herein include, but are not necessarily limited to, the following shown in Table 3:

Table 3

Derivatives of JF10

AMINO ACID SEQUENCE	ICAM-1 SEQUENCE POSITION ACCORDING TO SIMMONS (1988)
PRANLTVVLLRGEKELKREPAVGEP	[117-141]
RANLTVVLLRGEKELKREPAVGEP	[118-141]
ANLTVVLLRGEKELKREPAVGEP	[119-141]
NLTVVLLRGEKELKREPAVGEP	[120-141]
LTVVLLRGEKELKREPAVGEP	[121-141]
TVVLLRGEKELKREPAVGEP	[122-141]
VVLLRGEKELKREPAVGEP	[123-141]
RGEKELKREPAVGEP	[127-141]
LKREPAVGEP	[132-141]
GGAPRANLTVVLLRGEKEL	[114-132]
TVVLLRGEKEL	[122-132]
LLRGEKEL	[125-132]
LRGEKEL	[126-132]

Amino acid analogues of JF13A contemplated herein include, but are not necessarily limited to, the following shown in Table 4:

Table 4

DERIVATIVES OF JF13A

AMINO ACID SEQUENCE	ICAM-1 SEQUENCE POSITION ACCORDING TO SIMMONS et al. (1988)
RKIKKYRLQQAQKGTPMKPNTQATPP	[482-507]
KKYRLQQAQKGTPMKPNTQATPP	[485-507]
RLQQAQKGTPMKPNTQATPP	[488-507]
AQKGTPMKPNTQATPP	[492-507]
TPMKPNTQATPP	[496-507]
KPNTQATPP	[499-507]
TQATPP	[502-507]
ATPP	[504-507]

The functional region of ICAM-1 represented by peptides such as JF7B, appears to be distinct from the adhesion sites that have been identified (Fecondo et al., 1991; Fecondo et al., unpublished) JF7B has no inhibitory effect upon adhesion in all systems so far tested and, hence, the effect of the ICAM-1-like peptides contemplated herein may be independent of the "normal" LFA-1/ICAM-1 interactions.

The present invention also contemplates a method of inhibiting infection of cells by a retrovirus in a mammal comprising administering to said mammal an effective amount of an ICAM-1-like peptide or analogue thereof for a time and under conditions sufficient to inhibit, reduce and/or interfere with the retrovirus replication. Preferably, the mammal is a human and the retrovirus is HIV or its variants. In particular the HIV may be HIV-1 or HIV-2. Reference hereinafter to "HIV" includes all relevant retroviruses. By "inhibiting" HIV infection includes complete, substantially complete or only partial inhibition of infection. The term may apply to prophylaxis, i.e. before a human is exposed or during treatment, to inhibit the virus, or virus-infected cells, from infecting other cells. HIV infection is applied herein in relation to replication of the virus and syncytia formation since these parameters, up to the present time, represent the most convenient means to monitor infection. It is not the intention, however, to limit the definition of HIV infection to only these parameters since other indicators of infection may be substituted by one skilled in the art without departing from the scope of the present invention.

The method of administration will vary depending on the circumstances. Examples of such administration would include intravenous injection or infusion. Depending on the particular ICAM-1-like peptide or analogue used, administration by other routes, such as intranasal, oral, intraperitoneal, sub-cutaneous, rectal, topical or by any means whereby the active molecules can be put in contact with target cells and/or viruses may be possible. In these cases, the peptides or analogues may have to be modified to or co-administered with other molecules to prevent their breakdown or to prolong their half life or to facilitate entry into the bloodstream or target area.

The effective amount of ICAM-1-like peptide or its analogue will be that required to reduce, inhibit or interfere with HIV replication and must not be at cytotoxic levels or at least cause only clinically acceptable cytotoxicity. The actual concentration selected will vary depending on the exigencies of the clinical situation but will generally be greater than 0.005μM and preferably in the range 0.005-200μM. The inclusion of agonists to the ICAM-1-like peptides or their analogues in any therapeutic programme or the inclusion of other molecules having activity against HIV or in promoting the immune system, may result in less ICAM-1-like peptide or analogue being required. In such a case, 5nM to 100μM may be sufficient.

In accordance with this method, another active agent may be co-administered or sequentially administered to facilitate the treatment and/or activity of the ICAM-1-like peptides or analogues. Such other active agents include anti-viral agents, immune response stimulating agents, cytokines and analogues of ICAM-1-like peptides. In particular, the present invention extends to mixtures of two or more different ICAM-1-like peptides and/or their analogues.

Furthermore, microorganisms, such as normal flora organisms may be engineered to express appropriate amounts of peptide or analogue either as single molecules or as tandem or multiple repeats as discussed above. An example of a suitable microorganism is Escherichia coli. Such organisms and their use in administering the active peptides are encompassed by the present invention.

Another aspect of the present invention is directed to pharmaceutical compositions comprising an ICAM-1-like peptide and/or analogue and one or more pharmaceutically acceptable carriers and/or diluents. A convenient

reference for the preparation of pharmaceutical compositions including suitable carrier vehicles and their formulation, inclusive of other human proteins, eg. human serum albumin, is described in Remington's Pharmaceutical Sciences 17th ed., Mack Publishing Co., edited by Osol et al., which is hereby incorporated by reference.

The pharmaceutical compositions may also include additional molecules to stabilize the active agent or which act as agonists or otherwise assist the ICAM-1-peptide and/or its analogue to perform the desired function.

Furthermore, the present invention extends to the use of ICAM-1-like peptides and/or its analogues in the manufacture of a medicament for the treatment or prevention of HIV infection in humans.

The ICAM-1-peptide and/or its analogue may be in combination with another active agent which will also act against HIV or against the symptoms caused by infection of HIV. For example, ICAM-1-like peptides and/or analogues thereof may be used in combination with soluble CD4 or its derivatives or any other molecule capable of binding, associating and/or otherwise interacting with the HIV surface glycoprotein and/or transmembrane glycoprotein. Alternatively, the ICAM-1-like peptides and/or their analogues may be used in combination with HIV anti-viral compounds and/or a molecule capable of stimulating the immune system.

The present invention also extends to the use of ICAM-1-like peptides and/or analogues thereof to quantitatively or qualitatively detect or screen for LFA-1 receptors on cells in the blood stream or other body fluids. A range of techniques are available which could use the above

molecules directly or indirectly via antibodies to same, such as an ELISA or radioimmunoassay. The ICAM-1-like peptide and/or its analogue, may also be used in immobilized form to isolate specific LFA-1 expressing cells. The present invention, therefore, also extends to monoclonal or polyclonal antibodies to the ICAM-1-like peptides or their analogues.

The present invention is further described by the following non-limiting figures and examples.

In the Figures:

Figure 1 Analysis of the surface expression of LFA-1 (a and b) and ICAM-1 (c and d) on CEM (a and c) and MT-2 (b and d) cells. Classed matched control antibodies are shown by the dotted line.

Figure 2 Cytotoxicity testing with ICAM-1 derived peptides JF7B (a and d), JF9 (b and e) and JF13A (c and f) with CEM (a, b and c) and MT-2 (d, e and f) cells.

Figure 3 Inhibition of virus antigen production from CEM/HTLV-III_B (a, b and c) or MT-2/228200 (d, e and f) virus-infected cells or mock-infected cells (stripe) in the presence of peptides JF7B (a and d), JF9 (b and e) and JF13A (c and f) at 0 (blank), 10 (cross-hatch) or 100 (black) µg/ml.

Figure 4 Cell morphology of mock-infected (b) and HIV isolate 228200 infected (a and c) MT-2 cells at 8 days post-infection. Panel c is infected cells in the presence of 10µg/ml peptide JF7B. Magnification in all panels is x200.

Figure 5 Inhibition of virus antigen production from HIV isolate 228200 infected MT-2 cells in the presence of W-CAM-1 monoclonal antibody (10µg/ml) pre- (heavy stripe), post- (cross-hatch) and pre- and post-infection (black) compared to positive (blank), negative (heavy stiple) and antibody (heavy cross-hatch) controls.

EXAMPLE 1

MATERIALS AND METHODS

Source of Virus and Cells

The HTLV-I transformed cell line, MT-2, was established by Miyoshi et al. (1981), by the co-cultivation of cells from a patient with adult T-cell leukaemia with normal cord lymphocytes, and provided by Dr. Y. Hinuma, Institute for Virus Research, Kyoto University, Japan. CEM cells are a lymphoblastoid cell line derived by Foley et al. (1965) from an individual with acute lymphoblastic leukaemia. The prototype virus HTLV-III_B was obtained from Dr R Gallo, Laboratory of Tumour Cell Biology, National Cancer Institute, Bethesda, U.S.A. and isolate 228200 from a patient attending Fairfield Hospital. The characteristics of the local isolate are described elsewhere (Kiernan et al., 1990).

Synthesis of Peptides

Peptides were synthesized on an Applied Biosystems automated peptide synthesizer using highly optimized solid phase t-Boc chemistry protocols (Clark-Lewis and Kent, 1989). The peptides were deprotected and cleaved from the resin by S_N1-S_N2 acidolysis in HF according to the methods described by Tam et al. (1983). Peptides were purified to >95% by preparative reverse phase HPLC using an Aquapore C8 100 x 10 mm 20 micron Prep 10 cartridge column and analysed on Aquapore RP-300 30 x 4.6

mm 7 micron cartridge columns (Applied Biosystems Inc., Santa Clara, CA).

Flow Cytometry

For flow cytometry cells were incubated with anti-LFA-1 or Fluorescein isothiocyanate (FITC) labelled anti-ICAM-1 MAb followed by FITC-conjugated second antibody for the anti-LFA-1 MAb and analysed by flow cytometry.

Toxicity Testing

Testing for cytotoxicity was performed with all peptides by incubation of cells with test peptide and 1/2 volume medium changes every 2 days adding peptide back at the same concentration. Cell viability was assessed by trypan blue exclusion.

Inhibition of virus Replication with Synthetic ICAM-1-like Peptides

Purified peptides were solubilized in distilled water at 10 mg/ml and diluted in RPMI 1640 medium containing 29.2 µg/ml glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 2 µg/ml polybrene (polybrene used only for CEM cells) and 10% (v/v) heat inactivated foetal calf serum (RF-10) to working dilution. Cells (4×10^5 in 1ml) were pretreated for 1 hour at 37°C with peptide, followed by 1ml of virus at 1000 pfu (Kiernan et al., 1988) for 2 hours at 3°C in 5% CO_2. The cells were then washed 3 times with Eagle's Basal Medium, (BME) resuspended in RF-10 containing the test peptide and incubated for 8 days. Cells were fed every 2 days by 1/2 medium changes with RF-10 containing peptide. Cells were assessed for syncytium formation and virus antigen production (Innotest EIA, Innogenetics N.V., Belgium).

Inhibition with Monoclonal Antibody to ICAM-1

Cells (MT-2 cells at 400,000/ml) were pre-incubated for 1h at 37°C with W-CAM-1 antibody (Boyd et al. 1988) or control antibody (MOPC141) at a 1:500 dilution (10µg/ml) in RF-10. One millilitre of cell-free HIV supernatant (isolate 228200, 1000pfu) was added for 2h at 37°C. Virus and antibody were then removed by washing in Eagles BME. Cells were resuspended in fresh medium containing antibody as appropriate. Cells were fed by exchanging 1ml of supernatant fluid with fresh medium containing antibody on days 2, 4, 6 and 8 post-infection. Supernatant fluid was analysed for p24 using the DuPont assay according to the manufacturer's specifications (DuPont, Massachusetts, USA).

EXAMPLE 2

EFFECT OF ICAM-1-LIKE PEPTIDES OF HIV INFECTION

Choice of Synthetic Peptide Analogs

A total of 5 peptides were synthesized (Table 4). A peptide corresponding to the N-terminal of ICAM-1 was designated JF7B and peptide JFI3A corresponded to the C-terminal region of the ICAM-1 sequence. Based upon sequence comparisons (Pearson and Lipman, 1988) and hydrophobicity analysis (Kyte and Doolittle, 1982), a further 3 peptides were synthesized corresponding to internal sequences predicted to be surface-exposed regions (JF10 and JF11) and also a "unique" region (JF9) of the ICAM-1 sequence (Fecondo et al., 1991).

Expression of LFA-1 and ICAM-1 on the Surface of CEM and MT-2 Cells

The combination of HTLV-III infection of CEM cells and 228200 infection of MT-2 cells was chosen as excellent syncytium formation was observed in both cases with a

persistently infected population arising from the infection process with the former (Dalgleish et al., 1984) and total cytophathic effects observed with the latter (Kiernan et al., 1990).

Flow cytometric analyses of uninfected and HIV-infected CEM/HTLV-III_B and MT-2/228200 cells revealed CEM cells to have high expression of LFA-1 (Fig. 1a) opposed to MT-2 cells which had relatively low expression (Fig. 1b). In contrast to LFA-1, surface expression of ICAM-1 was very low for CEM cells (Fig. 1c) compared to MT-2 cells (Fig. 1d) where over 90% of cells expressed ICAM-1. The number of cells expressing ICAM-1 increased substantially (13 to 37%) after infection for CEM cells but did not change substantially for MT-2 cells as it was already at a very high level. The levels of expression were similar if different HIV isolates were used.

Effect of the Peptides on Uninfected Cells

Although there was no visible cytotoxicity observed with all five peptides tested we performed detailed toxicity testing with those that were inhibitory for HIV replication by incubation of uninfected cells with peptide concentrations ranging from 1 to 100 µg/ml and assessing viability (Fig. 2). All 3 peptides were cytotoxic at 1000 µg/ml (not shown). However, at lower concentrations of peptide (10µg/ml) the number of viable cells was greater than those with no peptide, particularly for peptides 7B and 13A (Fig. 2a, c, d and e). No toxicity was observed with any of the inhibitory peptides at 10µg/ml or lower concentrations according to the percentage of viable cells relative to the controls.

Effect of Peptides on Virus Replication

The five ICAM-like peptides were tested for their effect on HIV replication (Table 4). Three of these (JF7B, JF9 and JF13A) had an effect on HIV replication, with the concentrations tested (10 and 100 µg/ml), as observed by measuring antigen production in the supernatant over an 8 day period (Fig. 3.) Peptide JF7B showed the most marked inhibition with reduction in antigen production of 94% at 10 µg/ml and 99% at 100 µg/ml over the 8 day period tested with isolate 228200 infected MT-2 cells (Fig. 3d). Both peptides JF7B and JF9 showed consistent inhibition of HIV antigen production in both cell lines used with inhibition being dose dependent. Production of viral antigen recovered to almost normal levels with peptide JF9 at 10 µg/ml in 228200 infected MT-2 cells (Fig. 3e). Inhibition with peptide JF13A was not as marked, although dose dependent, with virus production recovering almost completely by day 8 with HTLV-III_B infected CEM cells (Fig. 3c) and in 228200/MT-2 infected cells at 10 µg/ml (Fig. 3f). Peptide JF11 consistently had little or no effect on virus replication or any morphological effects on either of the HIV-infected cell lines. All 5 peptides tested had little or no effect on antigen production when tested at 1 µg/ml.

Detailed observation of syncytium formation in both systems revealed no obvious differences in control and peptide treated HTLV-III_B-infected CEM cells, however syncytium formation with 228200-infected MT-2 cells revealed almost complete inhibition of formation of multinucleated giant cells compared with the positive control in the presence of peptides JF7B (Fig. 4a and c). A reduction of syncytium formation was also observed with peptide JF9 but it was not as marked. Interestingly, peptide JF7B caused the cells to clump, particularly at the higher concentration. Peptide JF13A also caused a

marked aggregation of the HIV-infected MT-2 cells at both 10 and 100 µg/ml.

Effect of W-CAM-1 Antibody on Virus Replication

On testing 228200 infected MT-2 cells with W-CAM-1 monoclonal antibody, which was known to markedly affect intercellular adhesion (Boyd et al, 1988), there was some effect on virus production (Fig. 5). Cells were exposed to antibody pre- and/or post-infection and assessed for virus production over an 8 day period. Treatment of cells pre- and post-infection gave the most consistent reduction in virus production but this was only modest compared to the reduction observed with ICAM-1 peptide 7B (0.3 $\log_{10}$OD compated to >1 $\log_{10}$OD; Fig. 5 compared to Fig. 3d).

Table 5

ICAM-1-LIKE PEPTIDES

Amino Acid No. of ICAM-1*	Peptide Sequence
1 to 23	NAQTSVSPSKVILPRGGSVLVTC (JF7B)
114 to 141	GGAPRANLTVVLLRGEKELKREPAVGEP (JF10)
367 to 394	VLYGPRLDERDAPGNWTWPENSQQTPMC (JF9)
415 to 439	PIGESVTVTRDLEGTYLCRARSTQG (JF11)
479 to 507	NRQRKIKKYRLQQAQKGTPMKPNTQATPP (JF13A)

* According to Simmons et al., 1988

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

REFERENCES

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Tam Proc. Natl. Acad. Sci. USA 85: 5409-5413, 1988

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Wawryk et al. 1989 Immunol Rev 108: 135-161

CLAIMS:

1. An ICAM-1-like peptide or analogue thereof having anti-viral properties.

2. The ICAM-1-like peptide or analogue thereof according to claim 1 having anti-retroviral properties.

3. The ICAM-1-like peptide or analogue thereof according to claim 2 wherein said peptide or analogue corresponds in whole or in part to one or more regions of ICAM-1 capable of, or responsible for, reducing, inhibiting and/or interfering with HIV replication.

4. The ICAM-1-like peptide or analogue thereof according to claim 3 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence in one or more of the amino acid regions 1 to 43, 460 to 507, 340 to 420 and/or 101 to 150 of ICAM-1.

5. The ICAM-1-like peptide or analogue thereof according to claim 4 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence in one or more of the amino acid regions 1 to 23, 367 to 394, 479 to 507 and/or 114 to 141 of ICAM-1.

6. The ICAM-1-like peptide or analogue thereof according to claim 5 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence:

NAQTSVSPSKVILPRGGSVLVTC.

7. The ICAM-1-like peptide or analogue thereof according to claim 5 wherein said peptide or analogue corresponds in whole or in part to the amino acid sequence:

VLYGPRLDERD[X]PGNWTWPENSQQTPMC,

wherein [X] is A or C.

8. The ICAM-1-like peptide or analogue thereof according to claim 7 wherein [X] is A.

9. The ICAM-1-like peptide or analogue thereof according to claim 5 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence:

GGAPRANLTVVLLRGEKELKREPAVGEP.

10. The ICAM-1-like peptide or analogue thereof according to claim 5 wherein said peptide or analogue corresponds in whole or in part to the amino acid sequence:

NRQRKIKKYRLQQAQKGTPMKPNTQATPP.

11. The ICAM-1-like peptide or analogue thereof according to claim 6 corresponding to one or more of the amino acid sequences listed in Table 1.

12. The ICAM-1-like peptide or analogue thereof according to claim 7 corresponding to one or more amino acid sequences listed in Table 2.

13. The ICAM-1-like peptide or analogue thereof according to claim 9 corresponding to one or more amino acid sequences listed in Table 3.

14. The ICAM-1-like peptide or analogue thereof according to claim 10 corresponding to one or more of the amino acid sequences listed in Table 4.

15. A method for inhibiting or preventing retroviral infection in a human comprising administering to said human an effective amount of an ICAM-1-like peptide or an analogue thereof for a time and under conditions sufficient to inhibit, reduce and/or interfere with retroviral replication.

16. The method according to claim 15 wherein the retrovirus is HIV or its variants.

17. The method according to claim 16 wherein said ICAM-1-like peptide or analogue thereof corresponds in whole or in part to one or more regions of ICAM-1 capable of, or responsible for, reducing, inhibiting and/or interfering with HIV replication.

18. The method according to claim 17 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence in one or more of the amino acid regions 1 to 43, 460 to 507, 340 to 420 and/or 101 to 150 of ICAM-1.

19. The method according to claim 18 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence in one or more of the amino acid regions 1 to 23, 367 to 394, 479 to 507 and/or 114-141 of ICAM-1.

20. The method according to claim 19 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence:

NAQTSVSPSKVILPRGGSVLVTC.

21. The method according to claim 19 wherein said peptide or analogue corresponds in whole or in part to the amino acid sequence:

VLYGPRLDERD[X]PGNWTWPENSQQTPMC,

wherein [X] is A or C.

22. The method according to claim 21 wherein [X] is A.

23. The method according to claim 19 wherein said peptide or analogue thereof corresponds in whole or in part to the amino acid sequence:

GGAPRANLTVVLLRGEKELKREPAVGEP.

24. The method according to claim 19 wherein said peptide or analogue corresponds in whole or in part to the amino acid sequence:

NQRKIKKYRLQQAQKGTPMKPNTQATPP.

25. The method according to claim 20 wherein the peptide or analogue thereof corresponds to one or more of the amino acid sequences listed in Table 1.

26. The method according to claim 21 wherein the peptide or analogue thereof corresponds to one or more amino acid sequences listed in Table 2.

27. The method according to claim 23 wherein the peptide or analogue thereof corresponds to one or more of the amino acid sequences listed in Table 3.

28. The method according to claim 24 wherein the peptide or analogue thereof corresponds to one or more of the amino acid sequences listed in Table 4.

29. The method according to claim 15 wherein the route of administration is the intravenous, infusion, oral, intranasal, intraperitoneal, subcutaneous, rectal or topical route or is secreted from an organism in the body.

30. The method according to claim 29 wherein the route of administration is the intravenous or infusion route.

31. The method according to claim 15 further comprising the co-administration or sequential administration of one or more other active agents.

32. The method according to claim 31 wherein the other active agents include anti-viral agents, immune response stimulating agents, cytokines and/or analogues of ICAM-1-like peptides.

33. A pharmaceutical composition comprising an ICAM-1-like peptide or analogue thereof according to any one of claims 1 to 14 and one or more pharmaceutically acceptable carriers and/or diluents.

34. The compositions according to claim 33 further comprising one or more other active agents.

35. The composition according to claim 34 wherein said active agents include anti-viral agents, immune response stimulating agents, cytokines and/or analogues of ICAM-1-like peptides.

36. The use of an ICAM-1-like peptide or an analogue thereof in the manufacture of a medicament for the treatment or prevention of retroviral infection in mammals.

37. The use according to claim 36 wherein the mammal is a human.

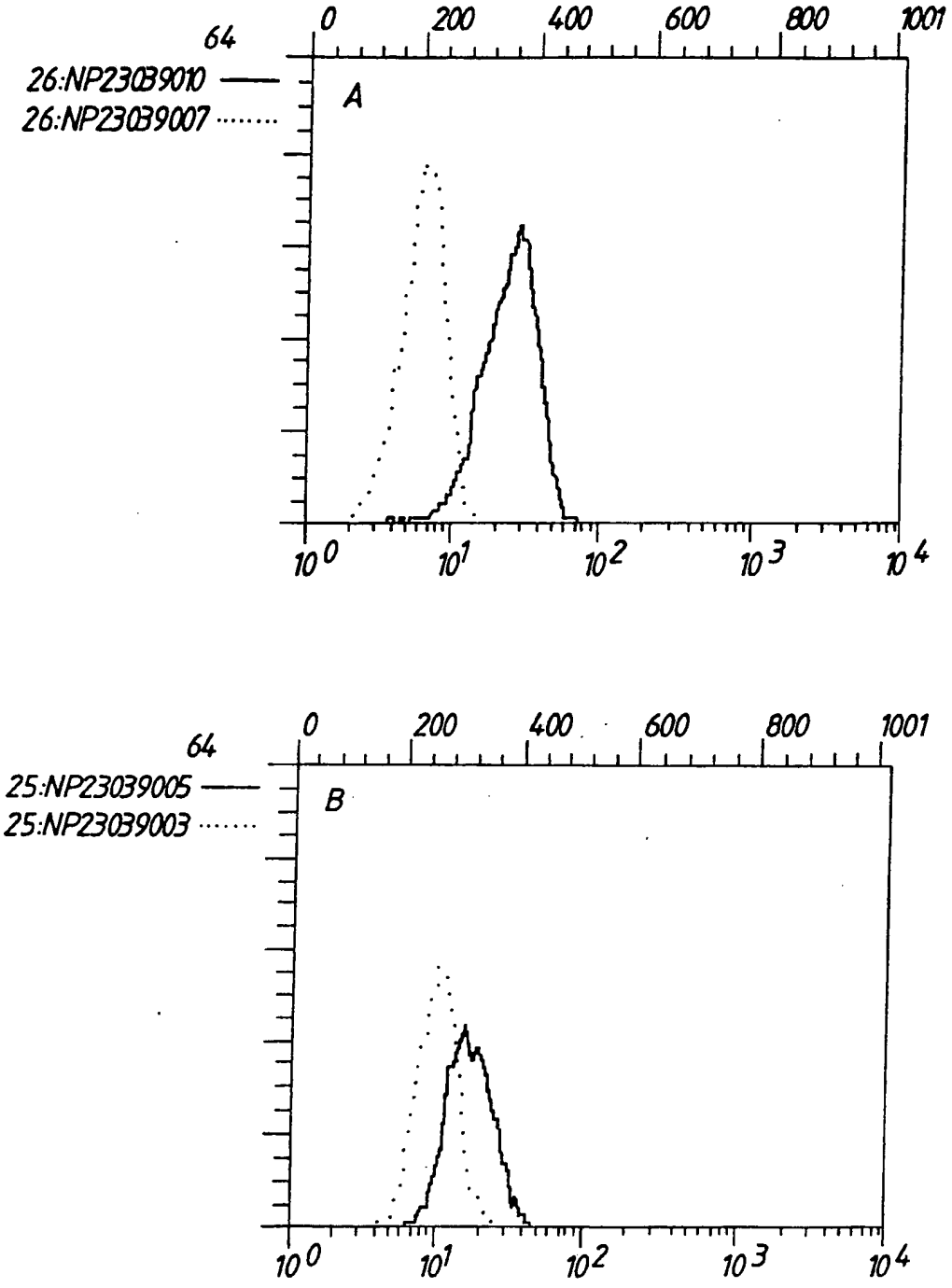
38. The use according to claim 37 wherein the retrovirus is HIV or its variants.

39. An antibody to the ICAM-1-like peptide or analogue thereof according to any one of claims 1 to 14.

40. The antibody according to claim 39 wherein said antibody is a monoclonal antibody.

41. The antibody according to claim 39 wherein said antibody is a polyclonal antibody.

1/8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64
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200
400
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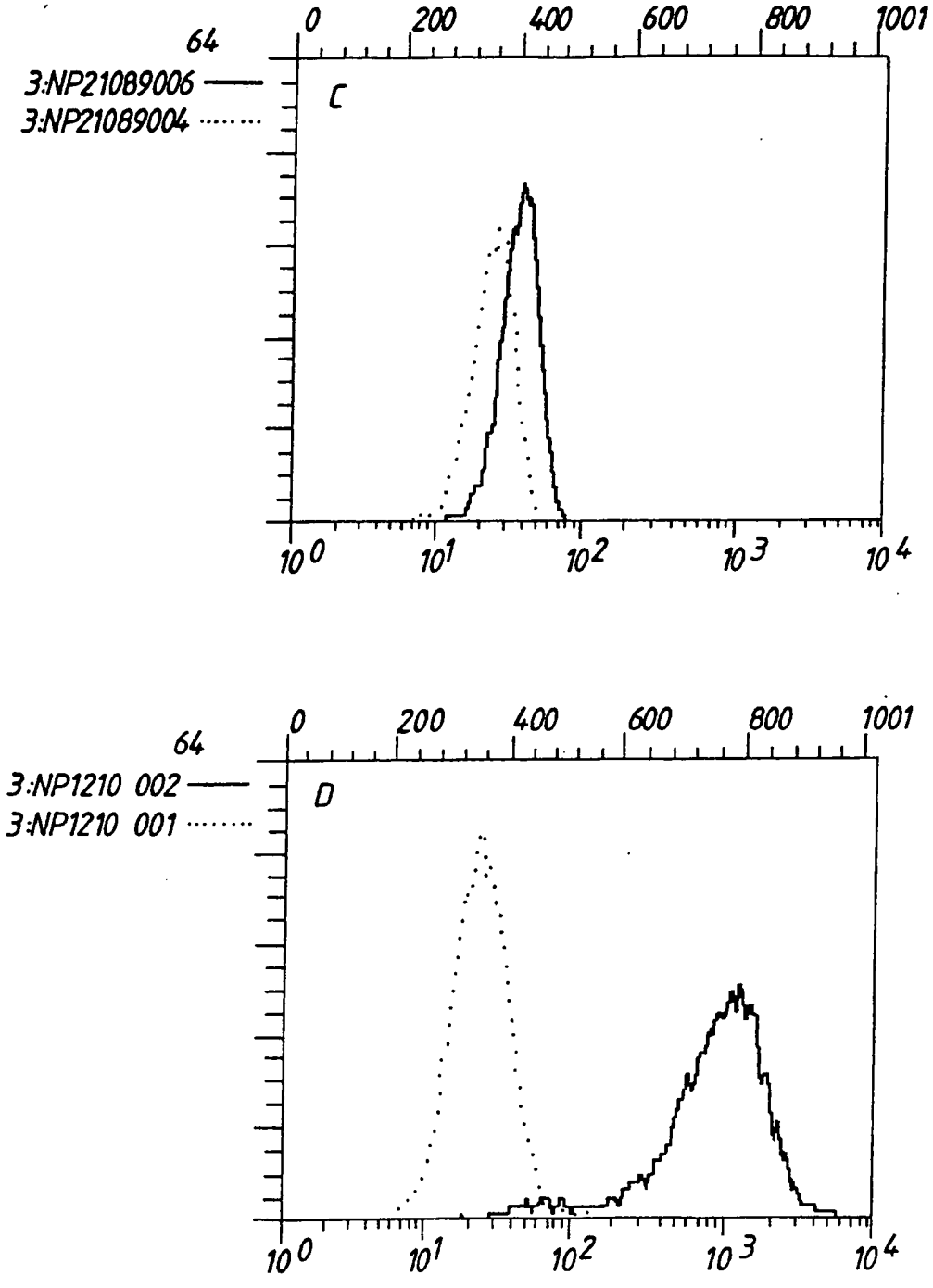
Fig.1(i).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10^4

Fig. 1(ii).

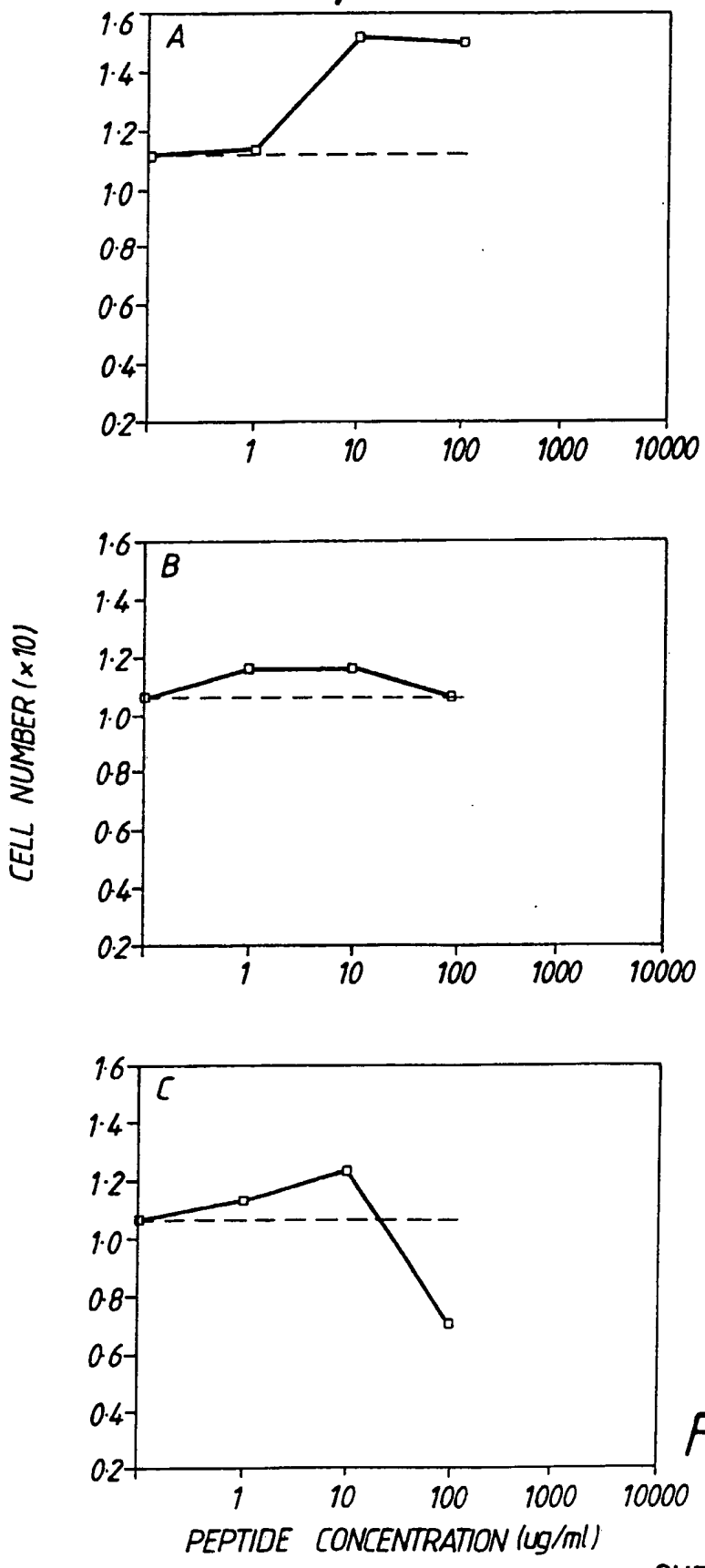
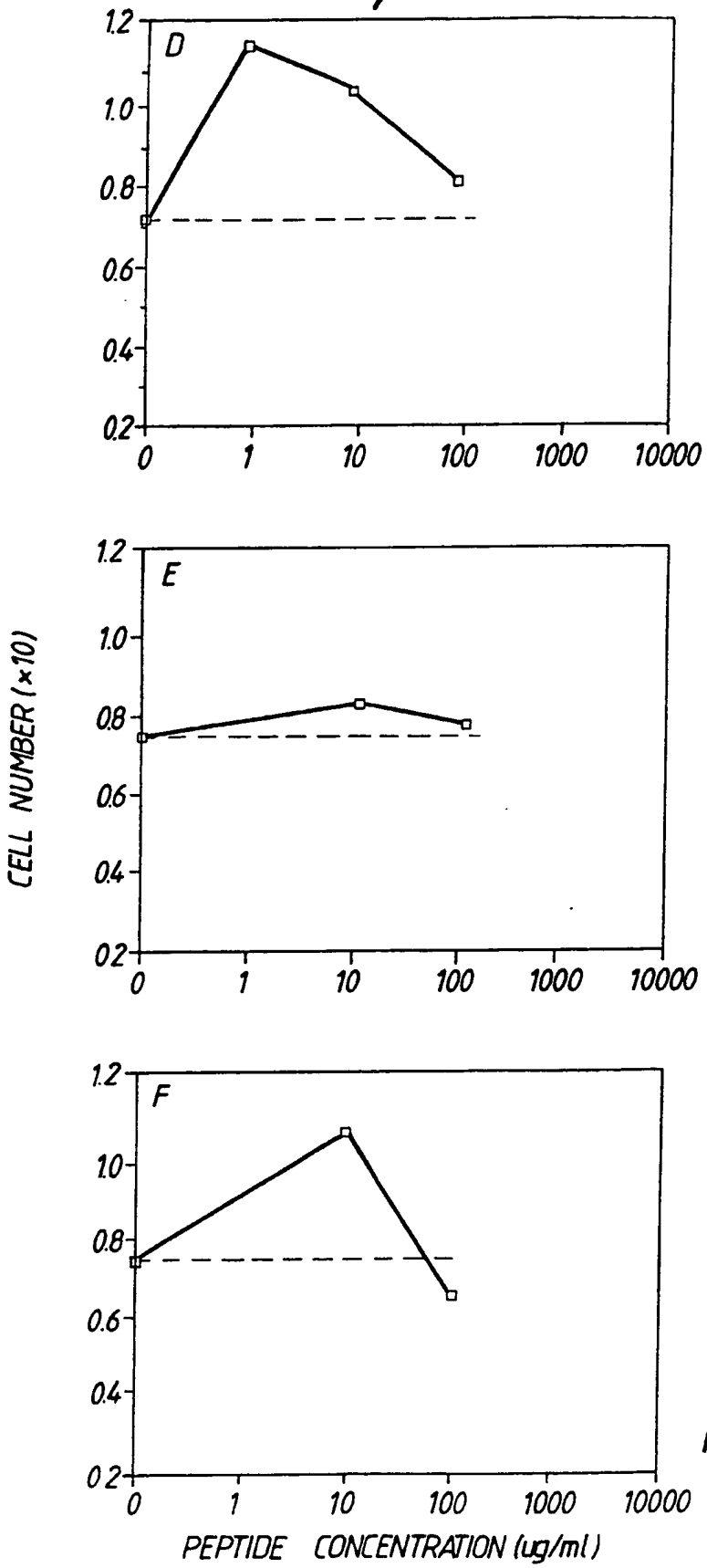

A
B
C
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
1
10
100
1000
10000
CELL NUMBER (×10)
PEPTIDE CONCENTRATION (ug/ml)

Fig.2(i).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E
F
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1.0
0.8
0.6
0.4
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1000
10000
CELL NUMBER (×10)
PEPTIDE CONCENTRATION (ug/ml)

Fig.2(ii).

5/8

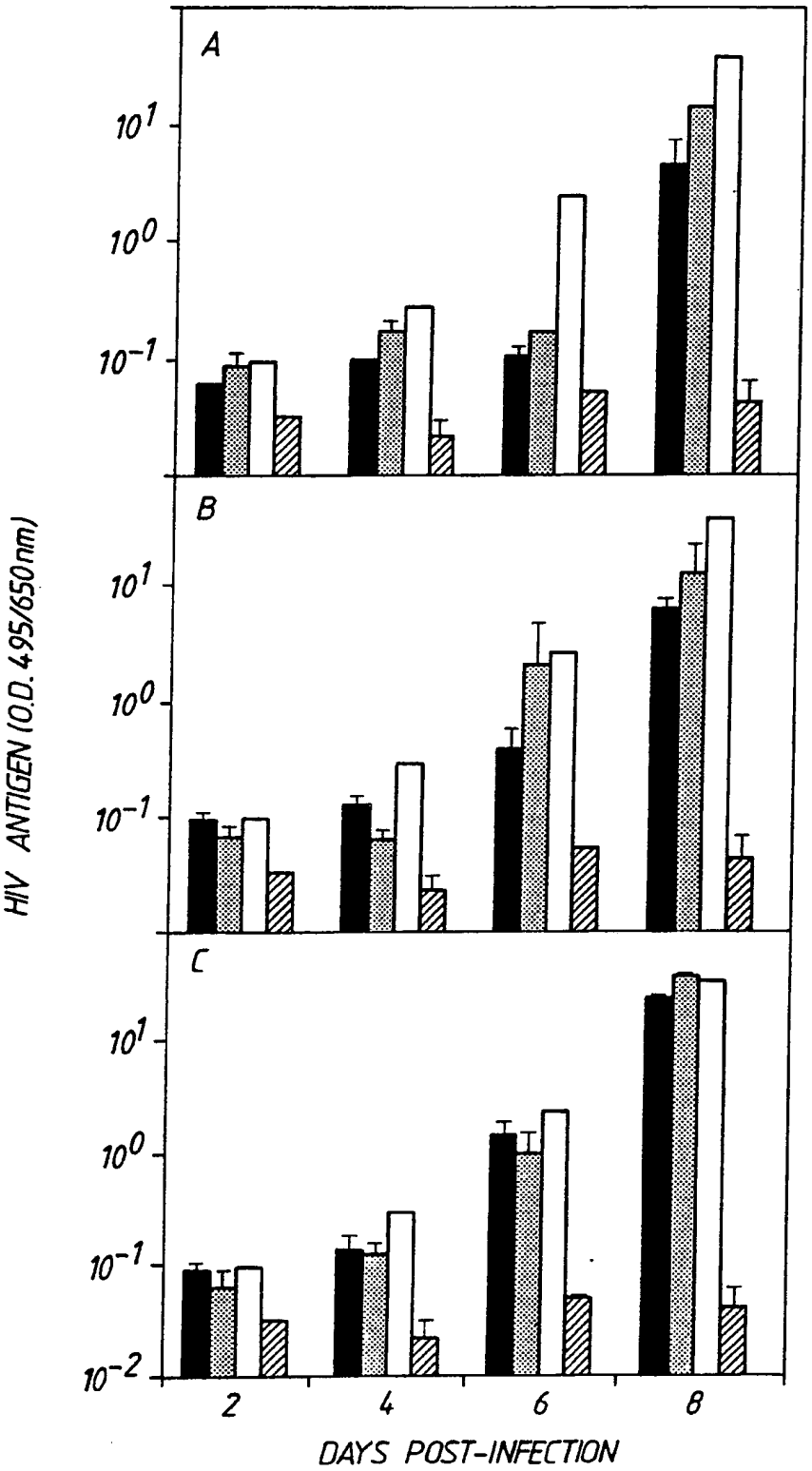
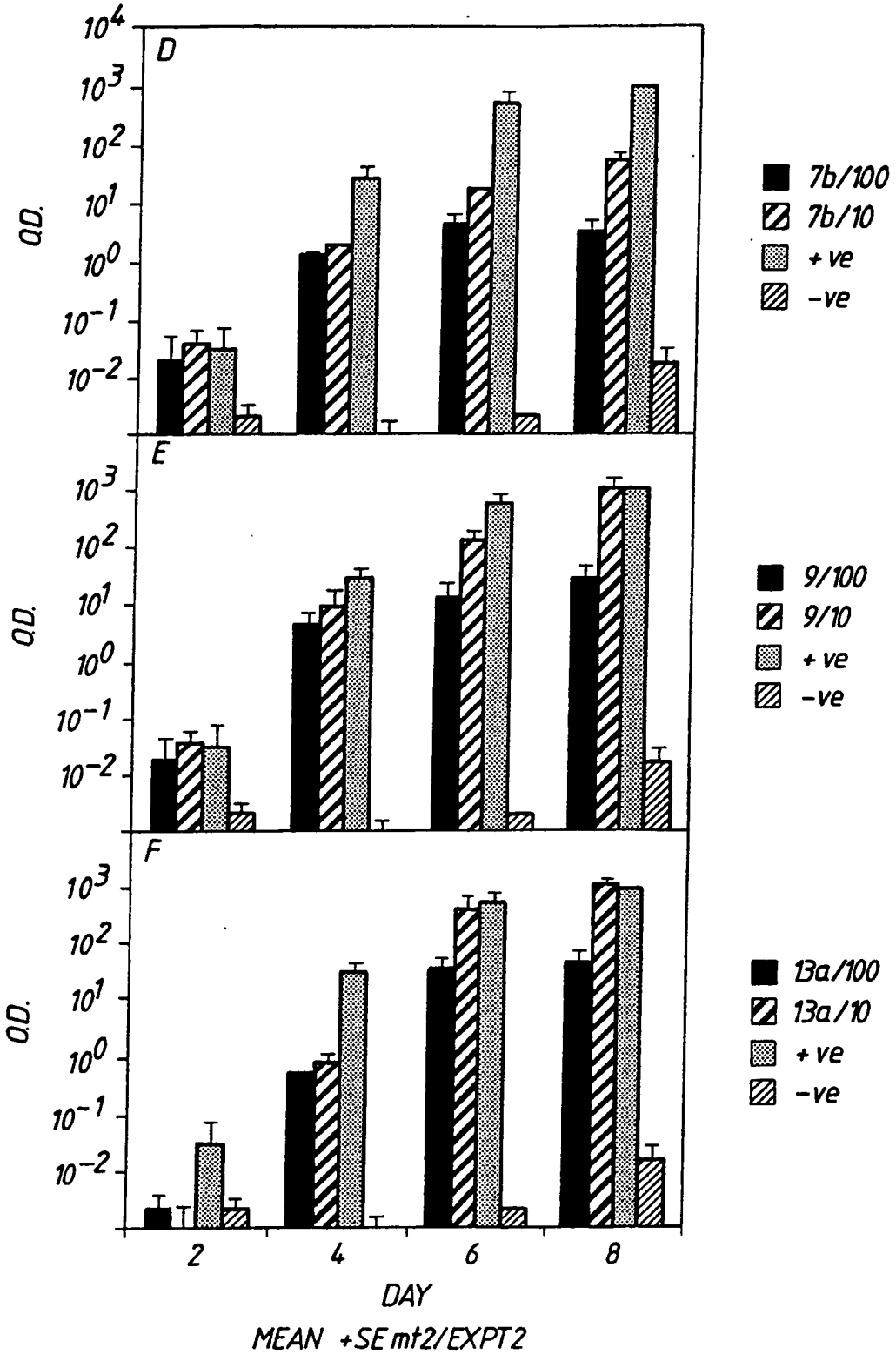

A
B
C
10^1
10^0
10^{-1}
10^{-2}
HIV ANTIGEN (O.D. 495/650 nm)
2
4
6
8
DAYS POST-INFECTION

Fig.3(i).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E
F
O.D.
10^4
10^3
10^2
10^1
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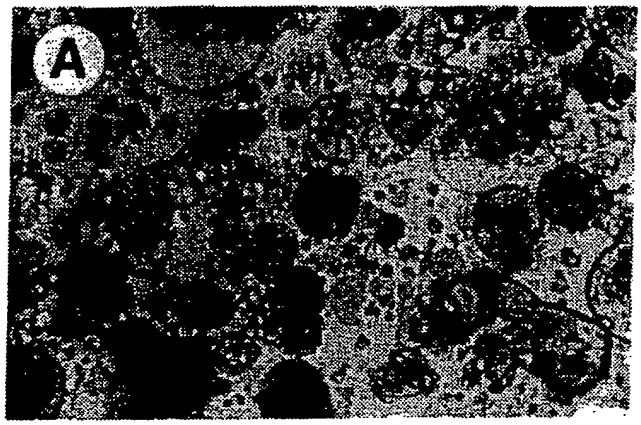
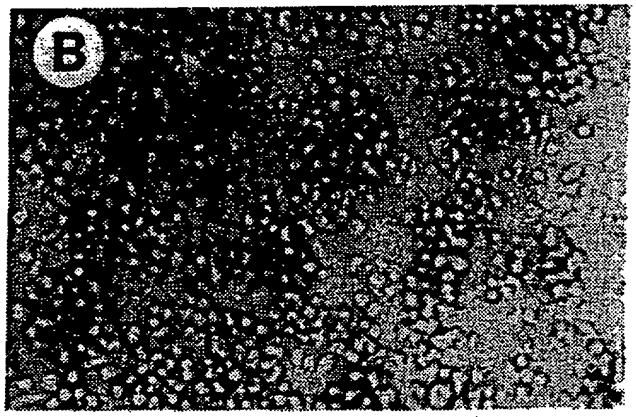
10^{-1}
10^{-2}
7b/100
7b/10
+ve
-ve
9/100
9/10
13a/100
13a/10
2
4
6
8
DAY
MEAN +SE mt2/EXPT2

Fig.3(ii).





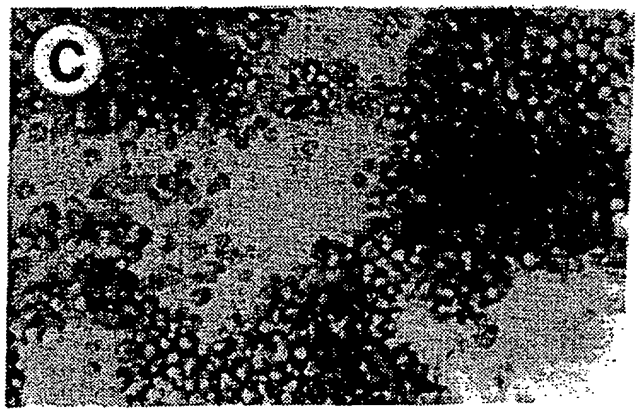
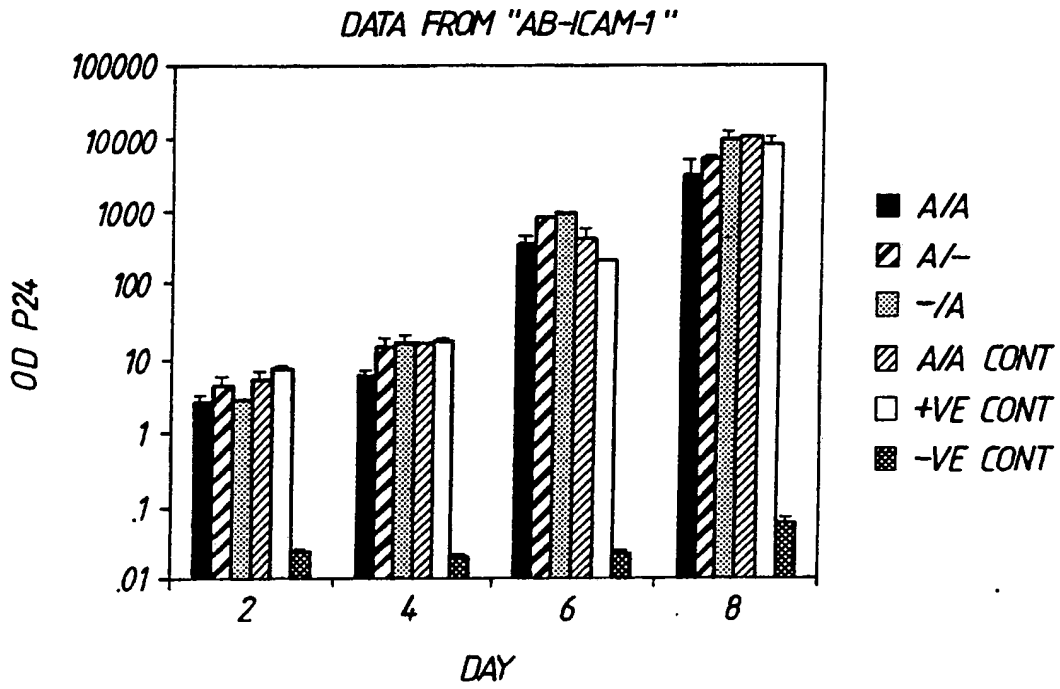


Fig.4.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10000
1000
100
10
1
.1
.01
OD P24
2
4
6
8
DAY
A/A
A/-
-/A
A/A CONT
+VE CONT
-VE CONT

Fig.5.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 91/00204

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C07K 5/06, 5/08, 5/10, 7/06, 7/08, 7/10, 13/00, 15/12, 15/14, C12P 21/08, A61K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
	DERWENT WPI/WPIL; CHEMICAL ABSTRACTS KEYWORDS: "intercellular ( ) ADHESION" or "ICAM:"

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

CHEMICAL ABSTRACTS : STN CAS-ONLINE PROTEIN SEQUENCE SEARCH
AU : C07K 5/06, 5/08, 5/10, 7/06, 7//08, 7/10, 13/00, 15/14, Co7C 103/52

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, [11] with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X	Journal of Immunology, vol. 137(4), pp. 1270-1274. 1986 (ROTHLEIN) "A Human Intercellular Adhesion Molecule (ICAM-1) Distinct From LFA-1."	(1, 2,33-35, 39-41)
X,Y	European Journal of Immunology, vol. 18, pp. 35-39. 1988 (DOUGHERTY et al) "The Function of human intercellular adhesion molecule-1 (ICAM-1) in the generation of an immune response."	(1,2, 33-35, 39-41)
X	Cell, vol. 52, pp. 925-933, 1988 (STAUNTON et al) "Primary Structure of ICAM-1 demonstrates Interaction between members of the Immunoglobulin and Integrin Supergene families" especially page 931.	(1-6, 6,9,11,13)

(continued)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
3 September 1991 (03.09.91)	9 Septembes 91
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	A.W. BESTOW

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Journal of Immunology, vol. 144(3), pp. 934-937, February 1, 1990 (VALENTIN et al) "The Leukocyte adhesion glycoprotein CD18 participates in HIV-1 induced syncytia formation in monocytoid and T cells."	(1-3, 15-17, 36-41)
P,X	Journal of Virology, vol. 64(6), pp. 2582-2587, June 1990. (LINEBERGER et al) "Antibodies that block Rhinovirus attachment Map to Domain 1 of the Major Group Receptor".	(1-3, 15-17, 39-41)
P,Y		(29-38)
P,X	Aids Research and Human Retroviruses, vol. 7(1) pp. 45-53, 1991. (GRUBER et al) "Re-evaulation of the Involvement of the Adhesion Molecules ICAM-1/LFA-1 in Syncytia formation of HIV-1-infected subclones of a CEM T-Cell Leukemic Line".	(1-3, 15,16, 39-41)
P,Y		(29-38)
X	AU,A, 15509/88 (BAYLOR COLLEGE OF MEDICINE & BOEHRINGER INGELHEIM PHARMACEUTICALS, INC.) 27 July 1989 (27.07.89).	(1,36,37,39-41)
X	AU,A, 15518/88 (DANA FARBER CANCER INSTITUTE), 10 NOVEMBER 1988 (10.11.88) entire document, especially tables 5,6,,8,9.	(1-6, 9,11,13, 33-35, 39-41)
X	AU,A, 26332/88 (DANA FARBER CANCER INSTITUTE), 18 May 1989 (18.05.89)	(1-6, 9,11,13, 33-35, 39-41)
X	AU,A, 26333/88 (BAYLOR COLLEGE OF MEDICINE & BOEHRINGER INGELHEIM PHARMACEUTICALS, INC.) 27 July 1989 (27.07.89)	(1,36,37,39-41)
X	AU,A, 29473/89 (THE GENERAL HOSPITAL CORPORATION) 22 September 1989 (22.09.89) especially pages 19, 91-96	(1-3, 33-44, 39-41)
X	AU,A, 40271/89 (MOLECULAR THERAPEUTICS, INC.) 8 March 1990 (08.03.90)	(1, 33-35, 39-41)
X	AU,A, 44128/89 (DANA FARBER CANCER INSTITUTE) 18 April 1990 (18.04.90)	(1-6, 9,11,13, 33-35, 39-41)
P,X	AU,A, 48767/90 (MOLECULAR THERAPEUTICS, INC.) 2 August 1990 (02.08.90)	(1-5, 10,14)
P,X	AU,A, 51294/90 (CENTER FOR BLOOD RESEARCH) 20 September 1990 (20.09.90) especially pages 6-10, 18, 21-25, 28, 61-67.	(1,2, 15-17, 29-41)
P,X	AU,A, 51299/90 (DANA FARBER CANCER INSTITUTE) 20 September 1990 (20.09.90).	(1-6, 9,11,13, 33-35, 39-41)
P,X	AU,A, 55532/90 (BAYLOR COLLEGE OF MEDICINE) 29 November 1990 (29.11.90)	(1,2, 15-17, 29-41)
P,X	AU,A, 55682/90 (BAYLOR COLLEGE OF MEDICINE) 29 November 1990 (29.11.90)	(1,2, 15-17, 29-41)
X	Chemical Abstracts, Registry No. 134448-23-0 STN CAS-ONLINE REGISTRY FILE PROTEIN SEQUENCE.	(4,5,7,8,12)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00204

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 15509/88	DK 2393/88	EP 314863	JP 1135724
	ZA 8803160		
AU 15518/88	DK 2394/88	FI 882037	IL 86246
	JP 1110700	PT 87390	EP 289949
	US 4822104		
AU 26333/88	DK 2393/88	EP 314863	
AU 29473/89	EP 330191	HU 890958	HU 53672
	WO 8908114		
AU 44128/89	AU 51299/90	DK 674/90	DK 1289/90
	EP 365837	FI 901318	FI 902490
	HU 54204	HU 56120	JP 3501861
	JP 3157397	NO 902311	WO 9003400
	ZA 8907335	AU 53499/90	EP 387701
	WO 9010453	WO 9010453	AU 40144/89
	EP 364690	WO 9010652	WO 9010646
	DK 4124/89		
AU 48767/90	EP 379904	FI 900331	IL 93119
	JP 3081296	NO 900155	
AU 40271/89	DK 4312/89	EP 362531	FI 894065
	IL 91454	JP 2238892	NO 893373
	PT 91570		

END OF ANNEX /CONTINUED

Patent Document Cited in Search Report	Patent Family Members		
AU 55682/90	WO 9013281	ZA 9003258	
AU 51294/90	AU 51172/90	CA 2012125	EP 391088
	HU 56133	JP 3072430	EP 387668
	FI 901170		
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	FI 902490	HU 54204	HU 56120
	JP 3157397	NO 902311	WO 9003400
	AU 53499/90	EP 387701	EP 365837
	WO 9010453	AU 44128/89	DD 285611
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	AU 53499/90	WO 9010652	WO 9010646
AU 55532/90	WO 9013316	ZA 9003223	

END OF ANNEX